

# Leptin, Nutrition, and the Programming of Hypothalamic Feeding Circuits

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## Abstract

A large body of epidemiological data suggests that adverse early environments, including obesity during pregnancy or early postnatal life, are linked to an elevated prevalence of metabolic disease in adult offspring. The mechanisms underlying these effects are still poorly understood, but recent data from rodents provide insight into a potential role for the brain in this ‘metabolic programming.’ This review summarizes the developmental changes that have been observed in the hypothalamus in response to changes in the early nutritional and hormonal environment. It also discusses how resetting a diverse array of neuroendocrine systems may have long-term effects on the regulation of metabolism and energy balance.

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## Introduction

The incidence of obesity is increasing at alarming rate worldwide. This trend ominously predicts that parallel increases in the prevalence of diseases such as type 2 diabetes and metabolic syndrome will soon follow. Many risk factors for obesity have been long established and include both sociological and genetic factors. In addition, a number of human epidemiological and animal studies have indicated that neonatal nutrition and maternal environmental factors may also have long-term effects on obesity. Maternal obesity and diabetes during pregnancy increase the incidence of offspring obesity and associated diseases such as type 2 diabetes. Similarly, maternal malnutrition and low birthweight with accelerated newborn-to-adolescent weight gain also increase the risk for developing obesity and metabolic syndrome [for review see 1, 2]. These observations have led to the hypothesis that an adverse

intrauterine and/or early postnatal environment during critical window(s) of development can program an individual to be more susceptible to metabolic diseases during adult life. However, the mechanisms by which the neonatal environment causes lasting perturbations in the body's energy balance have not been fully elucidated. Brain development has long been known to be particularly sensitive to changes in the perinatal environment. A number of factors affect the development and subsequent function of the brain. These include stress hormones, sex steroids and, more recently, nutrients and metabolic hormones. This review attempts to summarize our current understanding of hypothalamic development within the context of metabolic programming.

### **Importance of the Hypothalamus in Regulating Feeding and Energy Balance**

Appetite, energy expenditure, and metabolism are carefully regulated by the central nervous system. Lesion experiments and recently developed neuroanatomical methods and mouse genetic models have proven to be of critical importance for clarifying the detailed organization of neuronal pathways involved in the regulation of feeding and energy balance [3]. These approaches have defined the circuitry in the hypothalamus that appears to be an essential part of the homeostatic regulation of the energy balance [3]. Primary importance has been assigned to neurons within the arcuate nucleus of the hypothalamus (ARH). The ARH has long been associated with obesity and contains neurons that respond directly to a variety of hormonal and nutrient signals, including leptin, insulin, ghrelin, amino acids, and glucose [3]. The ARH contains a collection of heterogeneous neuronal populations, some of which play essential roles in the regulation of energy balance. One subpopulation of arcuate neurons coexpresses neuropeptide Y (NPY) and agouti-related peptide (AgRP). These neurons promote feeding and are activated by appetite-stimulating signals, such as ghrelin. Another group of neurons in the ARH coexpresses  $\alpha$ -melanocyte-stimulating hormone (derived from the proopiomelanocortin, POMC, precursor) and cocaine and amphetamine-regulated transcript. These neurons promote weight loss and are stimulated by anorexigenic factors, such as leptin. Both AgRP/NPY and POMC neurons send extensive projections to other parts of the hypothalamus, including the paraventricular (PVH) and dorsomedial (DMH) nuclei of the hypothalamus, as well as the lateral hypothalamic area (LHA). All of these hypothalamic regions also play important roles in controlling feeding and energy balance. Projections to the PVH are of particular interest since this nucleus provides major inputs to brain stem regions that regulate autonomic functions and also contains hypophysiotropic neurons that regulate hormonal secretions from the anterior pituitary. Recent work suggests that extra-hypothalamic sites, such as the ventral tegmental area (in the midbrain) and the nucleus of the

tractus solitarius (in the brain stem), may also be crucial for homeostatic and nonhomeostatic control of energy balance [4].

### **Development of Hypothalamic Feeding Circuits**

The development of the hypothalamus begins with neurogenesis in the proliferative zone of the neuroepithelial cells lining the third cerebral ventricle. This involves divisions that generate progenitor cells that ultimately produce postmitotic neurons. These newborn neurons subsequently migrate laterally from the proliferative zone of the third ventricle to form the various nuclei and areas that constitute the hypothalamus. Birthdating studies in mice revealed that neurons located in key hypothalamic nuclei known to control energy homeostasis are born prenatally and within distinct temporal domains [Ishii and Bouret, unpubl. data; for rat studies see 5]. The neurons found in the dorsomedial nucleus and in the LHA are born between embryonic day (E) 12 and E14. The ARH and ventromedial nucleus (VMH) exhibit a relatively long neurogenic period. Many neurons are born as early as E12, but some are generated as late as E16. In contrast, the paraventricular nucleus shows a short neurogenic period restricted to E12. It is largely unknown when specific hypothalamic cell types are born. Gene expression studies indicated that neurons in the ARH first express POMC mRNA on E12 [6], and NPY-immunoreactive cell bodies are found in the ARH as early as E14 [7]. The mRNA expression of both orexigenic (NPY, AgRP) and anorexigenic (POMC, cocaine and amphetamine-regulated transcript) neuropeptides continues to increase in the ARH during the postnatal period, reaching maximum expression levels by postnatal day 15 [8]. The extent to which hormonal factors influence these transcriptional changes is unknown, but recent studies suggest that the elevated levels of leptin that occur during postnatal life likely contribute to such patterns of gene expression [8–10].

Although neuronal proliferation occurs prenatally, hypothalamic neural connectivity is largely immature at birth and is primarily established postnatally. In part because of its importance for the regulation of feeding, the first systematic study used axonal labeling to define the ontogeny of neural projections from the ARH [11]. The results indicated that ARH neural projections innervate the DMH at P6, whereas innervation of the PVH and LHA occurs at P10 and P12, respectively. Moreover, these projections are not fully mature until the 3rd week of life, when pups begin to leave the microenvironment of the nest and search for solid food. Projections containing AgRP/NPY develop in a temporal pattern that generally matches the development of neural projections from the ARH [12]. Whether ARH neurons containing anorexigenic peptides (such as POMC neurons) extend their axons at the same time as those containing orexigenic peptides (e.g. AgRP/NPY) remains unknown. It is, however, intriguing that both NPY and melanocortin can modulate food

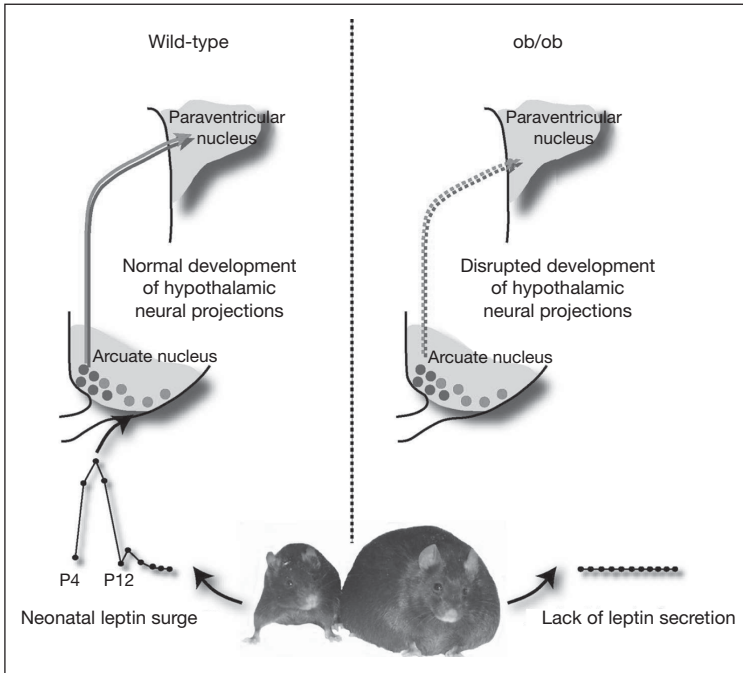
intake before ARH projections become fully mature [10, 13]. These observations suggest that pathways other than ARH projections are responsible for weight and feeding regulation during early postnatal life. Because efferent projections from the DMH and VMH appear to develop prior to those from the ARH [11], it is likely that these neural pathways are the primary regulators of feeding and metabolism during early postnatal life.

Together, these data indicate two important neurodevelopmental periods, one in utero and the other postnatal, during which alterations in the neonatal environment may affect hypothalamic neurogenesis and axonal formation and have long-term consequences on feeding and metabolism.

## Factors Influencing Hypothalamic Development

### *Leptin*

Circulating hormones that affect the brain represent important signals reflecting peripheral energy status, and may therefore influence the development of central mechanisms that regulate food intake and bodyweight. In this regard, elevated levels of circulating leptin, originally reported by Ahima et al. [14], were shown to occur when animals need to maximize food intake to support growth and maintain high thermoregulatory metabolic rates to optimize survival [15]. These physiological requirements seem to contradict an anorectic role for leptin during early stages of postnatal development. Consistent with this hypothesis, treatment of rodents with exogenous leptin does not increase milk intake or metabolic rates until after weaning. However, leptin is transported across the blood-brain barrier during early stages of postnatal life [16], and its receptors are expressed and competent in the developing hypothalamus, particularly in the ARH [8, 16]. These startling observations suggest that leptin may possess different functions during neonatal brain development independent of energy balance regulation [1, 14]. Because the neonatal surge in leptin appears to coincide with the development of major hypothalamic feeding circuits [11], it has been postulated that neonatal leptin may influence the development of these circuits [17]. This hypothesis has recently been tested through neuroanatomical experiments performed in leptin-deficient (*ob/ob*) mice. Axonal labeling of ARH axons revealed that ARH neural projections are severely reduced in *ob/ob* mice and that leptin deficiency causes a significant delay in the formation of projections from the ARH to each major target nucleus [17] (fig. 1). For example, while numerous ARH axons innervate the PVH at P12 in wild-type animals, very few ARH axons were observed in this region in *ob/ob* littermates. Furthermore, disruption of the ARH pathways in *ob/ob* mice appears to be permanent, since even on P60, a stage considered as mature with respect to the regulation of energy homeostasis in mice, a lower density of ARH axons innervating each of the hypothalamic sites involved in the control of energy homeostasis was observed (such



**Fig. 1.** Neurotrophic actions of the adipocyte-derived hormone leptin on hypothalamic feeding circuits. Elevated leptin levels are distinctly observed during the first 2 weeks of postnatal life in rodents. Instead of regulating food intake and bodyweight, the neonatal leptin surge appears to be an important trophic factor for the development of hypothalamic feeding circuits, and is critical for normal energy balance and hypothalamic regulation later in life. Consequently, mice lacking leptin (*ob/ob* mice) display abnormal development of neural projections from the arcuate nucleus to the paraventricular nucleus of the hypothalamus. In addition, leptin appears to exert its maximal effects on axonal formation during a restricted postnatal period.

as the PVH, DMH and LHA). Similar to the most important developmental factors, leptin seems to act primarily during a restricted postnatal critical period, which coincides with the naturally occurring surge in leptin [17]. The precise limits of this period of maximal sensitivity to the developmental activity of leptin remain to be defined, and it is still possible that prenatal exposure to leptin may influence other neurodevelopmental events, including neurogenesis or development of nonhypothalamic circuits. It will also be important to identify the molecular factors that mediate leptin's actions on hypothalamic development. A recent study used microarray analysis to study a set of genes that are specifically enriched in the VMH during postnatal development [18]. The identification of *Satb2* as a VMH gene regulated exclusively by leptin

during postnatal development has provided evidence that this transcription factor might be involved in the leptin pathway that is active during hypothalamic development.

The physiological relevance of the postnatal leptin surge has been supported by several observations. First, neonatal leptin treatment causes a long-term reduction in food intake in *ob/ob* mice [17]. Second, inhibition of the endogenous surge of leptin in normal rats results in increased susceptibility to the development of diet-induced obesity during adulthood [19]. Third, premature leptin surges (either prenatally acquired or induced by leptin supplementation in normal newborns) have lasting consequences on weight gain, glucose homeostasis, and leptin sensitivity [20]. Together, these data indicate that the correct timing and amplitude of the postnatal leptin surge appear to be required for normal energy balance regulation and that alterations in leptin levels during critical periods of postnatal development may therefore have long-term effects on feeding and metabolism.

### *Maternal Nutrition*

As noted above, a plethora of rodent and human studies have suggested that intrauterine nutritional status affects the later metabolic fate of an individual. The developmental programming of hypothalamic neuroendocrine systems by the perinatal environment represents a possible mechanism by which increased energy intake during pregnancy predisposes the offspring to developing metabolic syndrome. For example, maternal obesity not only causes offspring obesity, but it also results in persistent changes in the expression of appetite-regulating genes in the hypothalamus [21]. Offspring of dams fed a 40% excess in nutrient intake during late pregnancy demonstrated a permanent increase in hypothalamic POMC mRNA expression when compared to control animals [21]. In addition, a maternal high-fat diet results in upregulation of galanin expression (another orexigenic peptide) in the PVH and orexin in the LHA [22]. In addition to altering gene expression, maternal overnutrition during pregnancy also affects central leptin sensitivity, as demonstrated by the attenuated levels of leptin-induced phosphorylation of the signal transducer and activator of transcription 3 (pSTAT3, a key leptin receptor intracellular signaling pathway) in the ARH of offspring born to dams fed with a high fat diet [23]. Considering the importance of leptin signaling in the development of hypothalamic neural projections [24], it is tempting to speculate that the reduced leptin signaling observed in offspring of obese dams also leads to abnormal organization of hypothalamic feeding circuits.

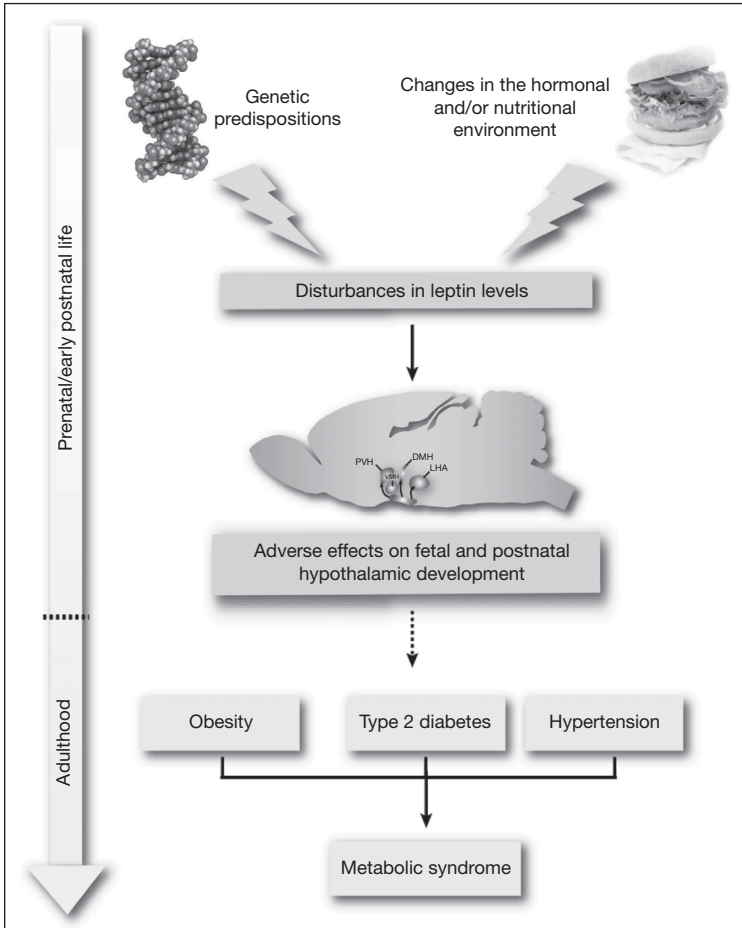
Other studies have investigated the importance of maternal undernutrition in metabolic programming. These studies revealed that severe maternal undernutrition sufficient to induce intrauterine growth retardation results in obesity in the offspring and has important effects on the expression of key hypothalamic genes involved in energy balance regulation. Specifically, down-

regulation of POMC mRNA levels was reported in the ARH and a reduction in POMC-immunoreactive fiber density was found in the PVH [25], indicating an overall reduction in the melanocortineric tone of animals born to undernourished dams. Similar to maternal overnutrition, undernutrition during pregnancy causes reduced central leptin sensitivity in the offspring [20]. The amount of proteins consumed during pregnancy also appears to influence appetite-related networks. Maternal protein restriction causes hypoinsulinemia in the offspring and is associated with increased NPY levels in the PVH and LHA at weaning [26]. Importantly, exposure to high doses of NPY during early postnatal development has been linked to permanent changes in food intake in adults [27].

Paradoxically, similar developmental and physiological outcomes are observed in offspring born to both under and overnourished mothers. Because manipulation of the maternal diet induces changes in various metabolic parameters, it may be difficult to determine which factor causes the adverse metabolic and neuroanatomical outcomes observed in the offspring. However, leptin represents a likely candidate for this metabolic programming. Indeed, neonatal leptin levels are tightly regulated by maternal nutrition [for review see 1, 2], and neonatal leptin therapy appears to reverse some of the deleterious consequences of maternal undernutrition [28]. However, whether neonatal leptin supplementation reprograms metabolism by affecting hypothalamic development and plasticity remains to be investigated.

### *Postnatal Nutrition*

Epidemiological studies comparing the outcomes of breastfeeding versus formula feeding provide clear evidence for the importance of early postnatal feeding in energy balance regulation in humans. Animal studies also confirmed the importance of early postnatal nutrition on later metabolism and have suggested that postnatal nutrition may also play an important role in programming the hypothalamic feeding systems (fig. 2). Using an animal model of divergent litter size, Plagemann [29] demonstrated that animals raised in small litters (three pups per litter) displayed increased bodyweight and adiposity during adult life. These metabolic abnormalities were associated with reduced expression of leptin receptor and increased mRNA expression of the orexigenic peptides NPY and AgRP in the ARH after weaning [30]. In addition, postnatally overnourished rats displayed abnormal responsiveness of VMH neurons to leptin: while leptin is a major stimulatory signal for VMH neurons in normal animals, it has mainly an inhibitory effect on VMH neurons in rats raised in small litters [31]. Postnatal overnutrition not only influences neuronal activity, but also affects the architecture of the neural circuitry involved in energy balance regulation. When compared to normally nourished animals, mice raised in small litters displayed a reduced density of ARH axons innervating the PVH [Bouret and Simerly, unpubl. data].



**Fig. 2.** Possible mechanisms by which the fetal and early neonatal environment may program adult metabolic diseases. The developing hypothalamus is highly sensitive to changes in the hormonal and nutritional environment. Thus, alterations in the perinatal environment or genetic predispositions can impact the development and subsequent function of hypothalamic circuits known to control feeding and energy balance. These effects appear to be mediated to some extent by abnormal leptin secretion or signaling during critical periods of fetal and postnatal development.

*Genetic Factors*

In addition to environmental perturbations, clear evidence now indicates that genetic factors may also influence the formation of neural pathways involved in energy balance regulation. The rodent model of diet-induced obesity (DIO) is particularly suitable for this area of research, as DIO rats



share various features with human obesity, including polygenic inheritance [32]. In outbred Sprague-Dawley rats fed a moderate-fat, high-energy diet, about one half develop DIO, whereas the remaining rats are diet resistant and gain no more weight than chow-fed controls [33]. Importantly, DIO rats exhibit central leptin resistance even before the animals are exposed to the high-energy diet [24]. The DIO genotype adversely influences neural development. Animals born to DIO dams display marked differences in the development of hypothalamic neural circuits that normally distribute leptin signals in the brain and are known to regulate energy homeostasis [24]. In vitro data suggest that the neurodevelopmental abnormalities observed in DIO rats are due to the reduced responsiveness of hypothalamic neurons to the trophic action of leptin that normally occurs during postnatal life [24]. Interestingly, early onset exercise in rats genetically predisposed to DIO ameliorates weight and adiposity gain as well as central leptin sensitivity for 10 weeks after exercise cessation [34]. These data suggest that the postnatal environment can override genetic factors that predispose an individual to obesity. These studies also emphasize the importance of prevention, particularly in juvenile populations with higher susceptibility to the development of obesity.

### Conclusions

Altogether, these studies indicate that functional hypothalamic neural systems may acquire their unique properties during restricted developmental periods under the influence of both genetic and environmental factors. There is very clear evidence that nutritional changes during critical periods of life can impact hypothalamic development and function, with lasting effects on feeding and metabolism (fig. 2). One of the key mechanisms for metabolic programming of the brain is leptin signaling during critical periods of fetal and postnatal development. As with most important developmental hormonal factors, neonatal leptin can be beneficial or detrimental to hypothalamic feeding pathways and later metabolism, depending on the timing and amplitude of action. It is likely that other environmental factors, such as exercise or stress, may also influence the development and function of neural systems involved in energy balance regulation, perhaps with cumulative effects when combined with metabolic factors. Approaches to minimize or reverse the consequences of such early life events may have therapeutic importance.

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## Discussion

*Dr. Gillman:* My question is both for the ob/ob model where you've administered leptin as well as the undernourished/overnourished litter size model. How much have you or others followed these animals up for phenotype for the later complications that you hypothesized might exist.

*Dr. Bouret:* Our group studied the long-term effects of neonatal leptin injections in ob/ob mice. We injected leptin into ob/ob mice from P4 to P16, then stopped the injections, and 3 weeks later we evaluated food intake and bodyweight. Interestingly, the ob/ob mice that were treated with leptin neonatally displayed a reduced food intake as compared to vehicle-treated pups [1]. Also, Vickers et al. [2] found that neonatal leptin injections in offspring born to prenatally undernourished rats also had long-term impact on energy balance regulation. So, it appears that neonatal leptin may have enduring and functional effects on feeding and energy balance regulation.

*Dr. Gillman:* And how about in the litter size-manipulated animals?

*Dr. Bouret:* We followed these animals until postnatal day 180, and found that both undernourished and overnourished animals displayed increased adiposity and abnormal glucose tolerance when they became adult. In addition, if animals raised in small litters are given a high-fat diet after weaning, they exhibit a higher sensitivity to diet-induced obesity as compared to normally nourished animals.

*Dr. Gillman:* I think it's great that you are looking at mechanisms and body composition and later outcomes because I think sometimes we stop at bodyweight and that sometimes gives us the right answer and sometimes it doesn't.

*Dr. Bouret:* You are absolutely right that being 'fat' or overweight is not necessarily a good indicator of obesity-related risks such as diabetes or cardiovascular disease. But

having more visceral fat as compared to subcutaneous fat, for example, may provide a better explanation of these risks. In this regard, the MRI is a great noninvasive tool to evaluate specific fat depots. Interestingly, our MRI analyses indicated that animals that were raised in small and large litters did not have any differences in subcutaneous fat, but displayed increased visceral fat that was accompanied by the development of diabetes.

*Dr. Lucas:* When you did the litter size manipulation experiment, you got a lovely gradation that you would expect in body size at follow-up and you got a very nice gradation in leptin response, and yet when you looked at the hypothalamus the undernourished and the overnourished groups produced a similar disorder. In other words, you didn't get a gradient of effect at the hypothalamic level. Can you explore that further, that was sort of rather unexpected.

*Dr. Bouret:* The data on hypothalamic development of both overnourished and undernourished animals can indeed be surprising because we may think that if leptin deficiency has a negative impact on hypothalamic development, then high levels of leptin may be beneficial for hypothalamic development. However, it appears that it's not just having leptin or not that is important for proper hypothalamic development, but it's rather to have the correct amount of leptin at the correct time. Not enough leptin is not good, but too much leptin is not beneficial either because it can create, for example, early leptin resistance. Also, two recent papers indicated that not only the correct amplitude, but also the correct timing of the postnatal leptin surge are required for normal regulation of energy homeostasis in later life [3, 4].

*Dr. Wainaina:* I wanted you to comment on the thrifty theory because in my country we have children who are undernourished in their early life. They are less sensitive to obesity and if they are able to take more in the early life, they survive better. The fact that they are able to eat the little that is available, they eat better and they survive better. Later on in life, when they become adults they have enough food. In my country, at the moment 10% of the adults have type 2 diabetes. What message do I carry on from here?

*Dr. Bouret:* It is difficult to give a simple answer to this question and, according to the talks we have heard this morning, it seems that birthweight is not the only determinant of obesity risk in adult life. The amount of weight that an individual gains during development is another important determinant for obesity and diabetes risks. So the message can be to avoid rapid growth and weight gain during critical periods of postnatal development as long as it does not interfere with the patient's health.

*Dr. Mobarak:* I wonder if we could do a similar study in humans, like an RCT. If we plan to do an RCT with human neonates, what sort of ethical issues could we face.

*Dr. Bouret:* It is very important for a basic science researcher to consider any implication of his research for public health. However, there is still a considerable amount of research to be done before applying our animal research to humans. In particular, there is an urgent need to better understand normal brain development in human beings and determine how it is comparable to rodents. For example, human brains may exhibit different periods of vulnerability as compared to rodent brains based on the temporal and regional maturation patterns of the hypothalamus. In addition, any interventions in kids or babies will require the use of noninvasive methods such as MRI. Equally important will be to convince parents that it is important to treat their babies even before they develop the disease as it is the case for perinatally acquired obesity.

*Dr. Domellöf:* Does leptin have this important trophic effect on hypothalamic neurons also in humans and, in that case, during which developmental period in the fetus or newborn?

*Dr. Bouret:* Unfortunately, not much is known about hypothalamic development and critical periods in humans. In rodents, it appears that the period of maximal

sensitivity for leptin starts during the embryonic life and ends around the first 2–3 weeks of life. In humans, it is believed that brain development is finished at birth, so one may think that the critical period for leptin's actions in humans may be restricted to late gestation. In fact, Dr. Lucas mentioned this morning that the environment during late gestation is more critical for brain development than the early postnatal life. However, the human hypothalamus may still be sensitive to neurotrophic cues after birth and remain relatively plastic until puberty. So I think that the key period for brain development in humans is during the embryonic life, but there are still opportunities for interventions until puberty.

*Dr. Ziegler:* My question is related to the previous one. Do human infants have a distinct leptin surge that you showed in mice and if yes, when does it occur?

*Dr. Bouret:* It is not clear whether humans also have a distinct leptin surge during neonatal life. One of the reasons is because there are many factors that can influence leptin secretion and that cannot be controlled in humans, so it's very hard to have accurate and reliable measures of leptin secretion in infants and fetuses. However, several papers reported, in humans, elevated levels of leptin in the mother's blood during gestation. In addition, the human placenta is also known to produce leptin, so the leptin produced by the mother and the placenta may act on the fetus to promote fetal development. In addition, it has been reported that leptin levels rise just before puberty in humans. We should also keep in mind that human milk also contains relatively high levels of leptin.

*Dr. Moelgaard:* Do you know anything about the interaction with any of the other hormones like choline or adiponectin?

*Dr. Bouret:* Of course, leptin is not the only hormone involved in hypothalamic development. Insulin, glucocorticoids, IGF as well as many other metabolic and non-metabolic hormones, may all play a very important role in brain development and plasticity. Also it's probably not a single hormone that is important for hypothalamic development but it's rather multiple hormones that act together and synergistically to promote hypothalamic development. .

*Dr. Lucas:* In the neonatal period in humans, there are quite a number of quite defined unmarked hormonal surges, testosterone, gut hormones and so forth. Are you saying that there isn't a leptin surge or that hasn't been looked at?

*Dr. Bouret:* Several papers have looked at leptin levels and reported elevated levels of leptin during both pre- and postnatal life in humans. However, there was no clear indication of distinct leptin surge, as it has been reported in rodents. It does not mean that there is no leptin surge in humans, it just means that it has not been detected yet.

*Dr. Lucas:* The importance of the question which I am sure that Dr. Ziegler was getting at is the extent to which the rat is a good model for humans in terms of early leptin physiology, but maybe there is a lot more to learn about that before we could throw a parallel.

*Dr. Bouret:* The issue of rodent versus human development is indeed very important. The general thinking is that brain development in humans is mostly complete at birth, in contrast to rodent brains, which continue to develop postnatally. However, these observations are primarily based on cortical and hippocampal development, and we still know relatively little on hypothalamic development in humans. Considerable amount of work needs to be done to determine exactly when the hypothalamus develops in humans and if it remains sensitive to neurotrophic cues during postnatal life.

*Dr. Lapillonne:* What is the trigger or what are the triggers of this postnatal surge of leptin?

*Dr. Bouret:* It is highly probable that multiple factors trigger the postnatal leptin surge. In particular, other hormonal signals, such as insulin, may influence neonatal

leptin levels. Another important factor that can trigger the leptin surge is maternal milk, and particularly the fat it contains.

*Dr. Cooke:* Mechanistically, how do you explain visceral adiposity in the undernourished and the overnourished rat pups?

*Dr. Bouret:* Here is my thinking: these animals have reduced leptin sensitivity, so it seems that both neonatally overnourished and undernourished animals develop some kind of leptin resistance which may contribute to this increased adiposity. Also, in addition to having an effect on the brain, postnatal nutrition may influence adipocyte proliferation/differentiation, which may also explain the increased visceral adiposity observed in overfed and underfed animals.

*Dr. Cooke:* To what extent do insulin and leptin interact, in that chronic hyperinsulinemia in rats may alter the pituitary adenoreceptor response and inhibit lipolysis.

*Dr. Bouret:* These animals are chronically hyperinsulinemic and have impaired ITT. Because insulin and leptin share common intracellular signal transduction pathways [5], it is possible that these two metabolic hormones may also act synergistically to mediate their neurotrophic effect.

*Dr. Cooke:* Which might explain the visceral adiposity because chronic hyperinsulinemia may downregulate visceral  $\beta$ -adrenoreceptor-mediated lipolysis [6].

*Dr. Bouret:* You are absolutely right. It is also important to note that the hyperinsulinemia is observed as early as the 1st week of life and persists throughout life.

*Dr. Hüppi:* Do you measure food intake in these animals, which is fairly difficult to do during the feeding, because that would relate to a change in the hormonal regulation or a change in anorexic or orexic behavior.

*Dr. Bouret:* We didn't measure food intake in our animals in part because it is technically very hard to get an accurate measure of food intake in mouse neonates and in mice in general. In our animal model of divergent litter size, pups have different access to food: animals raised in small litters have more food available as compared to animals raised in large litters. But the nutrients contained in the milk are the same in small and large litters. So it appears that the quantity of milk ingested during postnatal life is an important determinant of normal metabolic regulation in adult life.

*Dr. Cooke:* Just to follow through a little. Is there any interaction between leptin and ghrelin?

*Dr. Bouret:* I think that ghrelin is also a very important factor that influences appetite. However, the importance of ghrelin in early life programming of appetite is still poorly investigated. Nevertheless, it has been reported that ghrelin can have a trophic action on pancreatic cells, but we still don't know if ghrelin has similar trophic actions in the brain. In fact, we are currently investigating this hypothesis as well as studying potential synergetic effects between leptin, insulin and ghrelin.

*Dr. Singhal:* I just want to make a comment. In humans, there is evidence to suggest that in the 1st week of life there is no relationship between change in leptin and body mass index [7, 8]. Humans are probably leptin resistant early on, especially those born small for gestation, and this may stimulate rapid postnatal growth. So, I think that there are parallels between the human and animal data.

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